comparing said reference expression pattern with said target expression pattern to determine the function of said p53 sequence alteration.

REMARKS

The Invention

One embodiment of the invention relates to a method for detecting a functional mutation in a target up-stream regulatory gene. A reference sample is prepared from reference cells having a wild-type up-stream regulatory gene corresponding to the target up-stream regulatory gene. A target sample is prepared from target cells suspected of having a mutation in the target up-stream regulatory gene. The target cells are otherwise substantially similar to the reference cells. Expression of a plurality of more than 100 down-stream genes in the reference sample is detected to obtain a reference expression patter. The down-stream genes can be up-regulated or down-regulated by the wild-type regulatory gene. Expression of the plurality of down-stream genes in the target sample is detected to obtain a target expression pattern. The reference pattern and target expression pattern are compared to detect functional mutation in or inactivation of the target gene.

Another embodiment of the invention relates to a method for detecting a p53 gene functional mutation in target cells. A reference sample is prepared from reference cells having a wild-type p53 gene. The reference cells are otherwise substantially similar to the target cells. Expression of a plurality of more than 100 down-stream genes in said reference cells and said target cells is detected to obtain a target expression pattern and a reference expression pattern. The down-stream genes are up- or down-regulated by the wild-type p53 gene. The reference expression pattern and the target expression pattern are compared to detect the p53 functional mutation.

Still another embodiment of the invention relates to an in-cell functional assay for a p53 sequence alteration. A target sample is prepared from target cells having the p53 sequence alteration. A reference sample is prepared from reference cells having a wild-type p53 gene.

The reference cells are otherwise substantially similar to the target cells. Expression of a plurality of more than 100 down-stream genes in the reference cells is detected to obtain a reference expression pattern and in the target cells to obtain a target expression pattern. The down-stream genes are selected from the group consisting of p53 up-regulated genes and p53 down-regulated genes. The reference expression pattern and the target expression pattern are compared to determine the function of the p53 sequence alteration.

Amendments to the Claims

Independent claims 11, 29, and 34 are amended to recite "detecting the expression of a plurality of more than 100 down-stream genes." Support can be found on page 46, lines 3-7 which recites "[T]he expression of a subset of genes of interest in a diseased tissue is analyzed to obtain a diseased expression pattern . . . . The subset contains . . . more than 100 genes . . . ." Claim 34 is further amended to recite "said" thus correcting an obvious typographical error.

The Rejection of Claims 16, 17, and 29 Under 35 U.S.C. § 112, Second Paragraph

Claims 16, 17, and 29 stand rejected under 35 U.S.C. § 112, second paragraph as indefinite. This rejection is respectfully traversed.

The terms "expressed higher" and "expressed lower" in claims 16 and 17 are allegedly unclear because the claims do not recite what the higher or lower expression is compared to. Claims 16 and 17 clearly state what is being compared. In claim 16, one compares regulated genes of the target sample to the reference sample. In claim 17, one compares regulated genes of the target sample to the reference sample. Thus the relational terms higher and lower are clear because the claim defines what is being compared.

There are no occurrences of the phrases "expressed higher" or "expressed lower" in claim 29. Thus this ground of rejection does not apply.

Withdrawal of this rejection is respectfully requested.

The Rejection of Claims 11, 12, 14-16, 29-30, 32, 34-35, and 37 Under 35 U.S.C. § 103(a)

Claims 11, 12, 14-16, 29-30, 32, 34-35, and 37 stand rejected under 35 U.S.C. § 103(a) as unpatentable over Sanchez-Beato *et al.* (*J. Pathology*, 180:58-64, 1996, hereafter, "Sanchez-Beato") in view of Velculescu *et al.* (*Clinical Chem.*, 6:858-868, 1996, hereafter "Velculescu"). This rejection is respectfully traversed.

To establish a *prima facie* case of obviousness, a cited combination of prior art references must teach or suggest all of the claim limitations. MPEP § 2143.03, 8th ed., August, 2001.

Independent claim 11 is drawn to a method for detecting a functional mutation in a target up-stream regulatory gene. The method of amended claim 11 requires detecting the expression of more than 100 down-stream genes. Claims 12 and 14-16 are dependent from claim 11 and thus also require detecting the expression of more than 100 down-stream genes. Independent claim 29 is drawn to a method for detecting a p53 gene functional mutation in target cells. The method of amended claim 29 requires detecting the expression of more than 100 down-stream genes. Claims 30 and 32 are dependent from claim 29 and thus also require detecting the expression of more than 100 down-stream genes. Independent claim 34 is drawn to an in-cell functional assay for a p53 sequence alteration. The in-cell functional assay of amended claim 34 requires detecting the expression of more than 100 down-stream genes. Claims 35 and 37 are dependent from claim 34 and thus also require detecting the expression of more than 100 down-stream genes. Thus each of the rejected claims requires detection of more than 100 down-stream genes' expression.

Sanchez-Beato teaches a method to determine the status of a p53 gene in Hodgkin lymphoma cells. Sanchez-Beato teaches the status of the p53 gene in Hodgkin lymphoma cells is determined by measuring expression of two down-stream genes. "The pattern of expression of two downstream p53 proteins (MDM2 and $p21^{WAF1/CIP1}$) was studied as an indirect way of assessing p53 gene status." Page 58, abstract. Sanchez-Beato does not teach the positively

recited step of detecting the expression of more than 100 down-stream genes as recited in independent claims 11, 29, and 34.

Velculescu teaches the identity of twenty genes which are regulated by p53. "A . . . list of p53 target genes has been identified (Table 2 . . .)." Page 861, first column, lines 13-14. Table 2 lists eleven genes which are up-regulated by p53 and nine genes which are down-regulated by p53. The list includes MDM2 and $p21^{WAF1/CIP1}$ as taught in Sanchez-Beato. Velculescu does not teach the positively recited step of detecting the expression of more than 100 down-stream genes as recited in independent claims 11, 29, and 34.

Neither reference, alone or in combination, teaches or suggests the positively recited step of detecting the expression of more than 100 down-stream genes. Thus, a *prima facie* case of obviousness fails as neither reference alone or in combination teaches or suggests all of the claim limitations of claims 11, 12, 14-16, 29-30, 32, 34-35, and 37.

Withdrawal of this rejection is respectfully requested.

The Rejection of Claims 13, 31, and 36 Under 35 U.S.C. § 103(a)

Claims 13, 31, and 36 stand rejected under 35 U.S.C. § 103(a) as unpatentable over Sanchez-Beato *et al.* ("Sanchez-Beato") in view of Velculescu *et al.* ("Velculescu") and Brown *et al.* (U.S. 5,807,522, hereafter "Brown"). This rejection is respectfully traversed.

To establish a *prima facie* case of obviousness, a cited combination of prior art references must teach or suggest all of the claim limitations. MPEP § 2143.03, 8th ed., August, 2001.

Claim 13 depends from claim 12 which depends from independent claim 11 which is drawn to a method for detecting a functional mutation in a target up-stream regulatory gene. The method of amended claim 11 requires detecting the expression of more than 100 down-stream genes. The method of claim 12 further requires that the reference and target expression markers are detected by measuring the amount of transcripts of the down-stream genes in the reference

and target samples. The method of claim 13 further requires that the amount of transcripts is detected with a high density nucleic acid array. Claim 31 depends from claim 30 which depends from independent claim 29 which is drawn to a method for detecting a p53 gene functional mutation in target cells. The method of amended claim 29 requires detecting the expression of more than 100 down-stream genes. The method of claim 30 further requires that the expression of the down-stream genes is detected by measuring the amount of transcripts of the down-stream genes. The method of claim 31 further requires that the amount of transcripts is detected with a high density nucleic acid array. Claim 36 depends from claim 35 which depends from independent claim 34 which is drawn to an in-cell functional assay for a p53 sequence alteration. The in-cell functional assay of amended claim 34 requires detecting the expression of more than 100 down-stream genes. The method of claim 35 further requires that the expression of the down-stream genes is detected by measuring the amount of transcripts of the down-stream genes. The method of claim 36 further requires that the amount of transcripts is measured with a high density nucleic acid array.

Sanchez-Beato teaches a method to determine the status of a p53 gene in Hodgkin lymphoma cells. Sanchez-Beato teaches the status of the p53 gene in Hodgkin lymphoma cells is determined by measuring expression of two down-stream genes. "The pattern of expression of two downstream p53 proteins (MDM2 and $p21^{WAF1/CIP1}$) was studied as an indirect way of assessing p53 gene status." Page 58, abstract. Sanchez-Beato does not teach the positively recited step of detecting the expression of more than 100 down-stream genes as required by claims 13, 31, and 36.

Velculescu teaches the identity of twenty genes which are regulated by p53. "A . . . list of p53 target genes has been identified (Table 2 . . .)." Page 861, first column, lines 13-14. Table 2 lists eleven genes which are up-regulated by p53 and nine genes which are down-regulated by p53. The list includes MDM2 and $p21^{WAF1/CIP1}$ as taught in Sanchez-Beato. Velculescu does not teach the positively recited step of detecting the expression of more than 100 down-stream genes as required by claims 13, 31, and 36.

Brown discusses a method of forming a microarray. Column 6, line 65 through column 9, line 50. Brown does not teach p53 regulated genes, nor does Brown teach the positively recited step of detecting the expression of more than 100 down-stream genes as required by claims 13, 31, and 36. Thus Brown does not remedy the deficiency of Sanchez-Beato and Velulescu.

The references, alone or in combination, do not teach or suggest the positively recited step of detecting the expression of more than 100 down-stream genes. Thus, a *prima facie* case of obviousness fails as none of the references alone or in combination teaches or suggests all of the claim limitations of claims 13, 31, and 36.

Withdrawal of this rejection is respectfully requested.

The Rejection of Claim 17 Under 35 U.S.C. § 103(a)

Claim 17 stands rejected under 35 U.S.C. § 103(a) as unpatentable over Sanchez-Beato *et al.* ("Sanchez-Beato") in view of Velculescu *et al.* ("Velculescu") and Lewin (*Genes V*, 1994, page 76). This rejection is respectfully traversed.

To establish a *prima facie* case of obviousness, a cited combination of prior art references must teach or suggest all of the claim limitations. MPEP § 2143.03, 8th ed., August, 2001.

Dependent claim 17 depends from independent claim 11 which is drawn to a method for detecting a functional mutation in a target up-stream regulatory gene. The method of amended claim 11 requires detecting the expression of more than 100 down-stream genes. Claim 17 further requires indicating a gain-of-function mutation in the target gene if a significant portion of the down-regulated genes are expressed lower in the target sample than in the reference sample or if a significant portion of the up-regulated genes are expressed higher in the target sample than in the reference sample.

Sanchez-Beato teaches a method to determine the status of a p53 gene in Hodgkin lymphoma cells. Sanchez-Beato teaches the status of the p53 gene in Hodgkin lymphoma cells

is determined by measuring expression of two down-stream genes. "The pattern of expression of two downstream p53 proteins (MDM2 and $p21^{WAF1/CIP1}$) was studied as an indirect way of assessing p53 gene status." Page 58, abstract. Sanchez-Beato does not teach the positively recited step of detecting the expression of more than 100 down-stream genes as required by claim 17.

Velculescu teaches the identity of twenty genes which are regulated by p53. "A . . . list of p53 target genes has been identified (Table 2 . . .)." Page 861, first column, lines 13-14. Table 2 lists eleven genes which are up-regulated by p53 and nine genes which are down-regulated by p53. The list includes MDM2 and $p21^{WAF1/CIP1}$ as taught in Sanchez-Beato. Velculescu does not teach the positively recited step of detecting the expression of more than 100 down-stream genes as required by claim 17.

Lewin teaches that a mutation which causes "a protein to acquire a new function is called a **gain-of function** mutation." (Emphasis in original) Page 76, right column, lines 16-18. Lewin does not teach p53 regulated genes, nor does Lewin teach the positively recited step of detecting the expression of a plurality of more than 100 down-stream genes as required by claim 17. Lewin does not remedy the defect of the two primary references in failing to teach or suggest detection of expression of more than 100 down-stream genes.

Therefore the combination of references fails to teach or suggest all elements of the claim as required to make a *prima facie* case of obviousness. Withdrawal of this rejection is respectfully requested.

The Rejection of Claims 11-17, 29-32, and 34-37 Under The Judicially Created Doctrine of Obviousness-Type Double Patenting

Claims 11-17, 29-32, and 34-37 stand rejected under the judicially created doctrine of obviousness-type double patenting as unpatentable over claims 1-7 of U.S. 6,303,301 in view of Velculescu *et al.* (Velculescu").

A terminal disclaimer will be filed, if necessary, when the claims are in condition for allowance.

Respectfully submitted,

BANNER & WITCOFF, LTD.

Dated: 12-4-02

By: _____

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Appendix showing marked-up version of amended claim

11. A method for detecting a functional mutation in a target up-stream regulatory gene comprising:

preparing a reference sample from reference cells having a wild-type up-stream regulatory gene corresponding to said target up-stream regulatory gene;

preparing a target sample from target cells suspected of having a mutation in said target up-stream regulatory gene, said target cells being otherwise substantially similar to said reference cells;

detecting the expression of a plurality of more than [5] 100 down-stream genes in said reference sample to obtain a reference expression pattern, said down-stream genes being up or down regulated by said wild-type up-stream regulatory gene;

detecting the expression of said plurality of down-stream genes in said target sample to obtain a target expression pattern; and

comparing said reference pattern with said target expression pattern to detect functional mutation in or inactivation of said target gene.

29. A method for detecting a p53 gene functional mutation in target cells comprising the steps of:

preparing a reference sample from reference cells having a wild-type p53 gene, said reference cells being otherwise substantially similar to said target cells;

detecting the expression of a plurality of more than [five] 100 down-stream genes in said reference cells and said target cells to obtain a target expression pattern and a reference expression pattern, said down-stream genes being up- or down-regulated by said wild-type p53 gene; and

comparing said reference expression pattern with said target expression pattern to detect said p53 functional mutation.

34. An in-cell functional assay for a p53 sequence alteration comprising the steps of:

preparing a target sample from target cells having said p53 sequence alteration;

preparing a reference sample from reference cells having a wild-type p53 gene, said reference cells being otherwise substantially similar to said target cells;

detecting the expression of a plurality of more than [5] 100 down-stream genes in said reference cells to obtain a reference expression pattern and in [sad] said target cells to obtain a target expression pattern, said down-stream genes being selected from the group consisting of p53 up-regulated genes and p53 down-regulated genes; and

comparing said reference expression pattern with said target expression pattern to determine the function of said p53 sequence alteration.